Acaricidal Activity of *Juglans regia* Leaf Extracts on *Tetranychus viennensis* and *Tetranychus cinnabarinus* (Acari: Tetranychidae)

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ABSTRACT Leaf extracts of the walnut, *Juglans regia* L., were evaluated under laboratory conditions to determine their acaricidal activity on the mites *Tetranychus cinnabarinus* (Boisduval) and *Tetranychus viennensis* Zacher (Acari: Tetranychidae). Extracts had both contact and systemic toxicity to these mites. The four solvents tested for preparing crude extracts were petroleum ether, chloroform, ethyl acetate, and methanol. Methanol was the most efficient solvent, with an extraction rate from 17.06 ± 0.80 to 20.27 ± 0.28%. Petroleum ether was the least effective solvent, with extraction rates from 2.30 ± 0.13 to 2.71 ± 0.13%. However, the crude extracts with petroleum ether resulted in the highest mite mortality (79.04 ± 0.52%) in a slide dip bioassay. Mites mortalities from the concentrated extracts prepared by chloroform, ethyl acetate, methanol, or distilled water were significantly lower than petroleum ether. The mean lethal concentrations (LC_{50}) of the extracts from petroleum ether, chloroform, ethyl acetate, methanol, and distilled water to the two mite species were 0.73 ± 0.04, 1.66 ± 0.28, 4.96 ± 0.35, 7.45 ± 0.67, and 9.91 ± 0.32 mg/ml, respectively. After liquid chromatography and thin-layer chromatography, the concentrated extracts of petroleum ether were separated into eight fractions and tested for acaricidal activity. Fraction 6 produced significantly higher mite mortality rates than the other groups, killing ≈90% of both species.

KEY WORDS *Juglans regia*, *Tetranychus cinnabarinus*, *Tetranychus viennensis*, acaricidal activity

The spider mites *Tetranychus cinnabarinus* (Boisduval) and *Tetranychus viennensis* Zacher (Acari: Tetranychidae) are important pests of many agricultural crops and production trees in China and other parts of the world (Yigit and Erkilic 1992, Shi et al. 1994, Kielkiewicz 1996, Bhagat and Singh 1999, Gu et al. 2000). Both mite species have three to 15 generations per year in northern China (Wu et al. 2004, Li et al. 2004). They overwinter as adult females in the crevices of tree trunks, bark, and branches and in the ground litter. In apple (*Malus* spp.) orchards and crops in northern China, overwintering females initiate leaf feeding between late February and early March. Females oviposit on leaves in late March, and eggs hatch in early April (Wu et al. 2004, Li et al. 2004). Although both species typically do not kill host plants, heavy infestations may significantly reduce host plant vigor, resulting in leaf loss and reduced fruit yields (Shi et al. 1994, Pande et al. 1996, Shi and Liu 1996).

There are a number of species of natural enemies, including predatory mites, that normally maintain populations below damaging levels (Easterbrook 1992, Shi et al. 1994, Pratt et al. 2002, Prischmann et al. 2002). However, pesticides used to control other pests may inadvertently kill these beneficial insects. In addition, acaricides used to minimize the impacts of pest mites often are more toxic to their natural enemies, and they may actually aggravate the problem. The reduction of the natural enemy complex, coupled with the high reproductive potential and short life cycle of *T. cinnabarinus* and *T. viennensis*, can lead to the rapid development of outbreaks. These mites are also difficult to control due to their resistance to many commonly used pesticides (Cranham and Helle 1985, Grosscurt et al. 1988, Shi et al. 1994, Shen 1999, Landeros et al. 2002). Given the imposed quality restrictions on fresh market fruit (Li et al. 2004), new pesticides that are effective against phytophagous mites and that are also nontoxic to their natural enemies are needed to manage these mites in China.

Recently, an aggressive program designed to test native plants for potential acaricidal activities has been initiated in China, with >215 plants tested (G.L.S., unpublished data). Crude extracts of *Stellera chamaejasme* L. (Thymelaeaceae), a perennial poisonous weed found in meadows and young forest plantations throughout the temperate regions of Asia, demonstrated both contact and systemic toxicity to *T. viennensis* in slide dip tests (Shi et al. 2004). *Kochia Scoparia* (L.) Schrad, a cosmopolitan annual herbaceous plant, also exhibited both contact and systemic toxicity to *T. urticae*, *T. cinnabarinus*, and *T. viennensis*. In slide

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dip tests, extract fractions 66–69 from *K. Scoparia* provided 91.6% mean mite mortality after 24 h (Shi et al. 2006). *Wikstroemia chamedaphne* Meissn. (Thymelaeaceae), a perennial poisonous weed, and *Tageges erecta* L. (Compositae), an annual herbaceous plant, have also demonstrated great acaricidal activity (G.L.S., unpublished data).

Juglans regia L. (Juglandaceae) is a cosmopolitan tree species whose walnuts and leaves are used for medicinal purposes (Ma et al. 2006, Xu et al. 2006, Wang et al. 2006, Zhou and Lv 2006). Leaf extracts of *J. regia* have both insecticidal and bactericidal properties (Zhai et al. 2003, 2005; Liu et al. 2005). However, there are no published reports on the acaricidal properties of *J. regia.* The purpose of this study was to determine the potential acaricidal activity of *J. regia* leaf extracts on *T. cinnabarinus* and *T. viennensis.* Specific objectives were to identify any active fractions of the extracts and test four solvents for the preparation of concentrated extracts containing the active fractions.

Materials and Methods

Collection of Test Materials. *J. regia* leaves were collected randomly during core-hardening stage of fruit from a 10-yr-old orchard at the Shanxi Academy of Agricultural Science (112° 33′ 55″ E, 37° 53′ 04″ N, 820-m elevation), Shanxi Province, China. One-day-old adult females of *T. cinnabarinus* were obtained from laboratory rearing stock. Female *T. viennensis* were collected from heavily infested apple trees at the Shanxi Agricultural University Experimental Station (112° 32′ 45″ E, 37° 25′ 34″ N, 801-m elevation). This apple orchard was not treated with pesticides before collecting mites. All mites were placed on apple leaves and held in a 25- by 40-cm glass beaker under a photoperiod of 18:6 (L:D) h at room temperature (25 ± 3°C) and 50 ± 10% RH (Shi et al. 2004, 2006).

Crude Extraction Methods for *J. regia* Leaves. Fresh *J. regia* leaves were oven-dried at 45°C until the leaves were brittle. Preliminary tests conducted during the study suggest that oven drying does not affect acaricidal properties of *J. regia* leaves. Oven-dried leaves were ground to a fine powder. Four solvents, petroleum ether, chloroform, ethyl acetate, and methanol, were used to extract the active components of *J. regia* leaves. All the solvents were analytical grade (Beijing Chemical Reagents Company, Beijing, China). Two extraction methods were evaluated. The leaf powder was combined with a solvent in a 25-ml flask and either maintained at room temperature (25 ± 3°C) for 5 d or placed in a water bath at 40°C for 2 h. Each combination of extraction method and solvent was replicated three times, by using 20 g of powder of *J. regia* leaves and 100 ml of solvent for each replicate. The solvents were removed using a rotary evaporator (RE-52–98, Shanghai Yarong Biochemical Instrument Co., Shanghai, China), and the extracts were weighed (Shi et al. 2004, Shi et al. 2006). The extraction rate was calculated using extract weight (grams)/20 g of leaf powder × 100%.

Crude Extracts Solvent Partition of *J. regia* Leaves. The water bath immersion extraction method with petroleum ether as the solvent was selected for preparing the crude extracts for further concentration and partitioning. Twenty grams of extract obtained by this method was diluted in 200 ml of distilled water in a 500-ml separator funnel (Beijing Glass Factory, Beijing, China). Then, 200 ml of a partitioning solvent (petroleum ether, chloroform, ethyl acetate, methanol, or distilled water) was added to the funnel. The extracts were concentrated using a rotary evaporator and then weighed. The extraction rate was calculated as described previously. There were three replicates for each of the five solvents.

Isolation of Concentrated Extracts of *J. regia* Leaves to Determine Activity on *T. cinnabarinus* and *T. viennensis*. A 40- by 800-mm chromatography glass column (Beijing Glass Factory) was used to isolate components from the concentrated *J. regia* leaf extract. A mixture of 200 g of silica gel and 300 ml of petroleum ether were placed in the column, and then 5 g of the extract was added. A combination of petroleum ether, chloroform, ethyl acetate, and methanol (1:1:1:1) was slowly added to the column until all the concentrated extract eluted from the silica gel was collected at the bottom of the column into a 100-ml collection bottle. The mixture was separated into different components based on their polarites. In total, 108 fractions (bottles) were collected. Thin-layer chromatography (TLC) (model GF254, Tianjin Tianhe Medical Instruments and Apparatus Co., Tianjin, China) was used to compare constituents in each of the 108 fractions. Each fraction sample was partitioned into separate bands from the bottom to the top of the plate in TLC. Based on band appearance, the 108 fractions were pooled into eight groups. Each group was then evaporated to obtain concentrated isolates of the *J. regia* leaves.

Bioassays. The crude extracts of *J. regia* leaves obtained from the above-mentioned extractions (one extraction method with one solvent) were mixed with 20 μl of Tween 80 and diluted with distilled water at a concentration of 40 mg of powder/ml. The toxicity of the extracts on *T. cinnabarinus* and *T. viennensis* were tested using a slide dip technique. Thirty 1-d-old adult females were affixed to one side of double-sided adhesive tape attached to one end of a 10- by 2-cm glass slide. The end of the slide with the mites was dipped into each extract solution or distilled water (control) for 5 s. Once the slide was removed, any extra solution was absorbed with filter paper. Mortality was checked 24 h posttreatment (Shi et al. 2004, 2006).

Crude extracts collected using the water bath immersion method with a petroleum ether solvent were diluted with distilled water into a series of concentrations of 10, 20, 30, 40, and 50 mg of powder/ml and tested for acaricidal activity on *T. cinnabarinus* and *T. viennensis.* In addition to the slide dip technique described above, two additional bioassays were conducted to assess toxicity (LC_{50}) to mites. For the second bioassay, 30 adult females were placed on an

Table 1. Extraction rates of *J. regia* leaves by four solvents and two extraction methods, and percentage of mortality of the mites *T. cinnabarinus* and *T. viennensis* exposed to the crude extracts by using a slide dip method

Solvent	Extraction method[a]	Extraction rate (%) (mean ± SE)	Mortality (%) of *T. cinnabarinus* (mean ± SE)	Mortality (%) of *T. viennensis* (mean ± SE)
Petroleum ether	Water bath	2.71 ± 0.13a	79.48 ± 0.59a	76.53 ± 0.56a
	Room temp	2.30 ± 0.13b	81.58 ± 0.57a	78.58 ± 0.58a
Chloroform	Water bath	4.42 ± 0.08a	43.25 ± 1.06a	41.35 ± 1.15a
	Room temp	3.90 ± 0.21b	43.90 ± 0.56a	44.33 ± 0.13a
Ethyl acetate	Water bath	5.07 ± 0.06a	34.44 ± 1.33a	41.40 ± 0.91a
	Room temp	4.07 ± 0.14b	32.23 ± 1.62a	43.43 ± 1.22a
Methanol	Water bath	20.27 ± 0.28a	34.64 ± 0.68a	38.40 ± 0.99a
	Room temp	17.06 ± 0.80b	30.18 ± 0.86a	31.74 ± 1.03b

Mean values within a solvent in each column with the same letter are not significantly different ($P = 0.05$; Tukey's HSD test [SPSS Inc. 1999]).
[a] Water bath at 40°C for 2 h; room temp (25 ± 3°C) for 5 d.

apple leaf in a petri dish (10 by 2 cm) containing moist filter paper to prevent the leaf from drying. A circular barrier of moistened cotton (4 cm in diameter) was positioned around the leaf to prevent any mites from escaping. Each petri dish was placed inside the cylinder of a Potter spray tower (Burkard Agronomics; Uxbridge, Middlesex, United Kingdom) and sprayed with 1 ml of each extract concentration or distilled water. Mortality was recorded 24 h after treatment.

For the leaf bioassays to assess potential systemic activity, the leafstalk of an apple leaf was inserted into each extract concentration or distilled water for 72 h; then, it was placed in a petri dish with moist cotton to prevent the leaf from drying. Thirty adult females were placed on the leaf. A circular barrier of moistened cotton (4 cm in diameter) was positioned around the leaf to prevent the mites from escaping. Mortality was recorded after 48 h (Shi et al. 2004, 2006).

The leaf extracts concentrated by each of five solvents (petroleum ether, chloroform, ethyl acetate, methanol, or distilled water) were diluted with distilled water to obtain concentrations of 37.5, 75, 150, 300, 600, and 1,000 μg/ml. The acaricidal activity of these concentrations on *T. cinnabarinus* and *T. viennensis* was tested using the slide dip technique described above.

The concentrated isolates prepared by TLC were diluted with distilled water to a concentration of 500 μg/ml, and they were tested using the slide dip technique to determine which fraction(s) contained acaricidal compounds. Mortality was recorded at 24 h after treatment.

Each bioassay was replicated three times for each mite species, with 30 female adults per replicate. Treated mites were maintained at a photoperiod of 14:10 (L:D) h, 22–25°C, and 55 ± 15% RH. Mites were considered dead if they did not respond to a gentle probe with a fine brush.

Statistical Analysis. Differences among extraction rates within a solvent and among extraction rates of five concentrated extracts were compared by using a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test (SPSS Inc. 1999).

Mite mortality rates were corrected for control mortality by using Abbott's formula (Abbott 1925). Corrected mortality rates were normalized by an arcsine square-root transformation. The corrected mortality rates from the tests of the five concentrations of the crude extracts and from the six concentrations prepared using five different partitioning solvents were subject to a probit analysis POLO-PC (LeOra Software 1994) to estimate the LC_{50} (concentration at which 50% of the mites died) and the slope of the regression line. One-way ANOVAs followed by Tukey's HSD test ($P = 0.05$) were run to compare differences in mortality rates and LC_{50} between extraction methods, solvents, or concentrated isolates (SPSS Inc. 1999).

Results

Solvent and Leaf Extraction Methods for *J. regia*. The one-way ANOVA showed that both the solvent and extraction method had significant ($P < 0.05$) effects on the crude extraction rate of *J. regia* leaves. The solvent had a significantly greater effect than the extraction method based on the comparison of F-statistics (2876.2 and 253.7, respectively). Methanol was the most effective solvent (Table 1). The mean extraction rate for methanol under both extraction techniques (18.67%) was significantly higher than petroleum ether (2.51%) ($F = 185.28$, df = 3, $P = 0.0002$), chloroform (4.16%) ($F = 158.53$, df = 3, $P = 0.0005$), or ethyl acetate (4.57%) ($F = 138.49$, df = 3, $P = 0.0003$). Within a solvent, the water bath immersion technique was significantly more efficient than room temperature extraction based on extraction rates (Table 1).

Table 2. The LC_{50} of *J. regia* leaf crude extracts (petroleum ether as the solvent) for the mites *T. cinnabarinus*, and *T. viennensis* by using three different bioassay techniques

Bioassay technique	Mite species	Slope ± SE	LC_{50} (mg of power/ml) (95% CL)
Slide dip	*T. cinnabarinus*	2.34 ± 0.46	61.80 (56.1–65.8)c
	T. viennensis	2.79 ± 0.28	60.57 (57.8–64.4)c
Potter spray	*T. cinnabarinus*	1.41 ± 0.34	87.67 (83.2–91.1)ab
	T. viennensis	1.68 ± 0.28	86.83 (82.5–91.3)ab
Systemic activity	*T. cinnabarinus*	1.87 ± 0.38	97.80 (96.1–99.4)a
	T. viennensis	2.56 ± 0.73	95.10 (92.3–96.9)a

For all treatment and species combination, df = 3, $n = 6$. Means within a column followed by the same letters are not significantly different ($P = 0.05$; Tukey's HSD test, [SPSS Inc. 1999]).

Table 3. Percentage of yield of concentrated extracts of *J. regia* leaf crude extracts by using five different solvents and percentage of mortality rates of *T. cinnabarinus* and *T. viennensis* to the concentrated extracts by using a slide dip bioassay

Solvent	Yield (%) (mean ± SE)[a]	Mortality (%) of *T. cinnabarinus* (mean ± SE)[b]	Mortality (%) of *T. viennensis* (mean ± SE)[b]
Petroleum ether	80.01 ± 0.88a	81.65 ± 0.80a	83.70 ± 0.75a
Chloroform	11.00 ± 0.66b	25.80 ± 1.29b	24.33 ± 1.58b
Ethyl acetate	8.92 ± 0.40bc	17.55 ± 0.86c	16.11 ± 0.00c
Methanol	6.85 ± 0.52c	11.49 ± 0.59d	7.34 ± 0.00d
Distilled water	2.70 ± 0.21d	3.72 ± 0.58e	1.56 ± 0.00e

Means followed by the same letters are not significantly different ($P = 0.05$; Tukey's HSD test [SPSS Inc. 1999]).

[a] Yield (%) = wt concentrated extract/wt crude extract × 100.

[b] Mite mortality rate obtained using 3 mg of the concentrated extract per milliliter of distilled water.

Table 5. Mean percentage of mortality rates of the mites *T. cinnabarinus* and *T. viennensis* exposed to liquid chromatography fractions of *J. regia* leaves by using a slide dip bioassay

Isolate (fractions)	Mortality (%) of *T. cinnabarinus* (mean ± SE)	Mortality (%) of *T. viennensis* (mean ± SE)
0 (control)	0.00 ± 0.00e	0.00 ± 0.00d
1 (1–11)	2.23 ± 0.32e	2.48 ± 0.26d
2 (12–27)	55.28 ± 1.33b	55.84 ± 1.30b
3 (28–45)	9.41 ± 1.11d	4.78 ± 0.52d
4 (46–67)	33.50 ± 0.77c	31.23 ± 0.68c
5 (68–75)	1.94 ± 0.23e	2.32 ± 0.38d
6 (76–88)	90.99 ± 0.73a	89.97 ± 1.55a
7 (89–93)	36.79 ± 2.21c	37.21 ± 2.27c
8 (94–108)	3.47 ± 0.25e	2.56 ± 0.23d

Mite mortality was determined using 2 mg of extract fractions per milliliter of distilled water.

Means within a column followed by the same letters are not significantly different ($P = 0.05$; Tukey's HSD test [SPSS Inc. 1999]).

Activity of Crude Extracts of *J. regia* Leaves on *T. cinnabarinus* and *T. viennensis*. Petroleum ether extracts produced higher mortality rates for each mite species in the slide dip bioassay (Table 1), and combined mite mortality was significantly higher for this solvent (79.04%) than for the other three solvents ($F = 78.67$, df = 4, $P = 0.0007$). Within the solvents, only one significant difference in percentage of mite mortality was found between extracts prepared by the two methods (Table 1). Also, extraction by water bath immersion only required 2 h versus 5 d for room temperature immersion. Therefore the water bath immersion technique, using petroleum ether as the solvent, was selected for preparing crude leaf extracts for additional mortality tests and further concentration and partitioning.

The LC_{50} values for all three bioassay techniques suggested that *J. regia* leaves crude extracts possess both contact and systemic toxicity to the mites (Table 2).

Mortality of *T. cinnabarinus* and *T. viennensis* Exposed to Solvent Partitions of *J. regia* Leaf Extracts. Petroleum ether as the solvent produced significantly higher yields when partitioning the crude extracts and resulted in significantly higher mite mortality rates than the other four solvents tested (Table 3). Results from the bioassays also demonstrated that concentrated extracts produced using petroleum ether caused higher mite mortalities (>80%) than the other treatments. The LC_{50} values of the concentrated extracts from petroleum ether and chloroform to the two species combined or separate were significantly lower than those from the other solvents (Table 4). The LC_{50} values of concentrated leaf extracts prepared with ethyl acetate were significantly lower than those from distilled water.

Toxicity of *J. regia* Leaf Extract Fractions on *T. cinnabarinus* and *T. viennensis*. Based on band appearance in the TLC, 108 initial fractions were grouped into eight final fractions (Table 5). Mean mite mortality rates from fraction group 6 exceeded 89%, and they were significantly higher than for the other fraction groups. Three other fraction groups caused >30% mite mortality, whereas half of the groups produced mortality rates below 10%.

Discussion

Many plants possess pesticidal properties (Prakash and Rao 1997). Plant essential oils may exhibit fumigant or contact toxicity to mites (Lee et al. 1997, Choi et al. 2004). Rasikari et al. (2005) reported acaricidal activity of plant extracts from five subfamilies of Lami-

Table 4. Toxicity of concentrated leaf extracts of *J. regia* to *T. cinnabarinus* and *T. viennensis* by using a slide dip bioassay

Solvent	Mite	Slope ± SE	LC_{50} (mg of power/ml) (95% CL)	Combined LC_{50} (mg of powder/ml) for the two mite species (mean ± SE)
Petroleum ether	*T. cinnabarinus*	1.50 ± 0.063	0.79 (0.78–1.89)a	0.73 ± 0.04a
	T. viennensis	1.37 ± 0.047	0.67 (0.47–0.75)a	
Chloroform	*T. cinnabarinus*	1.32 ± 0.051	1.27 (1.03–3.53)a	1.66 ± 0.28a
	T. viennensis	1.52 ± 0.082	2.05 (1.74–5.22)a	
Ethyl acetate	*T. cinnabarinus*	0.93 ± 0.035	5.46 (4.69–7.33)b	4.96 ± 0.35b
	T. viennensis	0.91 ± 0.058	4.47 (3.76–4.98)b	
Methanol	*T. cinnabarinus*	0.84 ± 0.069	6.51 (5.86–7.18)b	7.45 ± 0.67bc
	T. viennensis	1.10 ± 0.051	8.40 (7.64–9.58)bc	
Distilled water	*T. cinnabarinus*	0.92 ± 0.034	9.45 (5.11–38.7)c	9.91 ± 0.32c
	T. viennensis	0.74 ± 0.023	10.36 (6.14–45.2)c	

For all treatment and species combination, df = 3, $n = 6$. Means within a column followed by the same letters are not significantly different ($P = 0.05$; Tukey's HSD test [SPSS Inc. 1999]).

aceae to *T. urticae.* However, in laboratory studies in China, <5% of all plants tested to date had extracts with acaricidal activity (G.L.S., unpublished data). We found that *J. regia* leaf extracts exhibited both contact and systemic toxicity to *T. cinnabarinus* and *T. viennensis* in the laboratory. It is suggested that the leaf extracts of *J. regia* had many special modes of action against mites at the same time, which suggested the advantages of botanical pesticides that reduce the potential of mite populations developing resistance. Further testing is required to determine whether the leaf extracts provide similar results on field populations of the mites.

Shi et al. (2004) determined the LC_{50} of concentrated extracts of *S. chamaejasme* prepared with chloroform was 0.23 mg/ml, compared with the LC_{50} of 0.71 mg/ml for *K. scoparia* extracts prepared with chloroform (Shi et al. 2006) and 0.73 mg/ml for *J. regia* leaf extracts prepared with petroleum ether in this study. The lethal concentrations could be lowered and/or differences in the required concentrations of the extracts among the three plants could be minimized if only the active components are used in an acaricide. The 9,12-octadecadienoic, *n*-hexadecanoic, and 9-octadecynoic acids were the main active components found in *K. scoparia* (G.L.S., unpublished data).

This study did not assess the source or the mode of action of the acaricidal activity of *J. regia* leaf extracts. Previous studies demonstrated that extracts of *J. regia* leaves have insecticidal properties (Zhang et al. 1999, 2000; Zhai et al. 2001). *J. regia* leaves contain terpenes, hydrocarbons, esters, and strong antioxidant components such as flavonoids and phenolic compounds (Buttery et al. 1986). Phenolic compounds, including tannins, flavanols, and flavonoids, are toxic to many bacteria, fungi, and insects (Olszewski 1954, Herrmann 1955, Miliauskas et al. 2004, Qadan et al. 2005). Juglone, another organic compound contained in the leaves, has been shown to have pesticidal and herbicidal properties (Duke and Ayensu 1985). When crushed, the leaves act as insect repellent (Uphof 1959, Usher 1974). Kaempferol and kaempferol-3-0-arafuranoside have been isolated from extracts of *J. regia* leaves, and these compounds have known allelopathic properties (Liu et al. 2004). Fractions 2, 4, 6, and 7 of the leaf extracts demonstrated some level of bioactivity against *T. cinnabarinus* and *T. viennensis.* Identification of the compounds present in the active fractions must be determined. The efficiency of varying extraction techniques and the identification of the active fractions obtained from this study should facilitate this investigation.

The development of an economical botanical acaricide is dependent on a reliable and consistent source of plant material. The ecological impacts of plant production and harvest, plus the toxicity of the product to mammals are other considerations. The wide distribution of *J. regia* (Zhai and Wei 1992, Ji 2003) and the use of its leaves in traditional Chinese medicine (Jiang et al. 2005) suggest that *J. regia* leaf extracts warrant further examination as a botanical acaricide. Phytotoxicity is also a concern (Rasikari et al. 2005), and any potential acaricide must be tested on the mite's host plants. The isolation and combination of active compounds from various plant extracts that have demonstrated acaricidal activity could increase the efficacy of novel miticides and reduce the potential of mite populations developing resistance.

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